Comprehensive Soil Microbial

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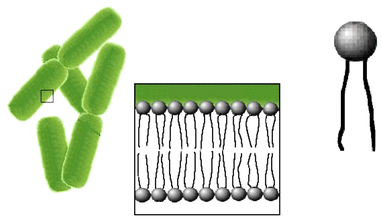
Abstract:

Analysis of the soil microbial communities at Ft. Benning, GA supported by the SERDP program “Indicators of Ecological Change” has brought together four research groups from three institutions to assess quantitative and qualitative measures of soil microbial biomass, community composition, nutritional status, and activity as reflections of military training impacts and its potential for reversibility. Methods for this work span the breadth of soil microbial ecology and include soil enzymes, DNA, phospholipid fatty acids (PLFA), community level carbon utilization profiles (CLCUP), and soil chemical analysis. Soil microbial biomass, respiration and enzyme activity rates are potential indicators of nutrient (e.g. C, N, P) storage / cycling and organic matter decomposition. Military activity causes loss of soil structure and organic carbon, reducing microbial and enzymatic activities. Results indicate a strong inverse relationship with the degree of soil disturbance, i.e. 87% of variance (ANOVA) can be explained by Total Phosphorus (TP) and Water Extractable Carbon levels, and 92% of variance by TP, Total Carbon, and Potentially Mineralizable Nitrogen (PMN). Attempts to understand the effects of disturbance as evaluated using the Biolog and FungiLog methods and soil nitrogen dynamics have focused along two watersheds and six sites that reflect three levels of disturbances (high, medium, and low) that are the result of training activities and forest management practices. Multi-year data are analyzed for long-term trends in soil microbial dynamics and functional diversity that might result from climatic and/or anthropogenic effects. Additional study of other training areas at Ft. Benning using soil PLFA profiles have been successful in building models to predict the amount of disturbance that a training site has received and have given insight into restoration processes. At other locations, a variety of DNA-based microbial indicators were evaluated, with genes representing nitrifying bacteria (ammonia monooxygenase; *amoA*) and the 16S rRNA gene of Type II methanotrophs being the most promising. Sequence analysis coupled with Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis revealed a tight correlation between one genotype of nitrifier closely related to *Nitrosomonas europea* and wetlands, although no correlation was observed with land quality. Individual phylotypes of Type II methanotrophs

Using Microbial Phospholipid Fatty Acids to Predict Disturbance

PLFA profiles reflects both the nature of the intracellular components and the extracellular environmental conditions.



Experimental Design:

Disturbance Intensity Gradient

Heavy **Moderate** **Light** **Control** **Remediated**

--Tank Maneuvers---
Turning in Neutral
Drive on Tank Trails

----Target Practice---
Heavy Artillery
Light Artillery

---Timber Harvest---
Clear Cut
Selective Thinning

Pine forest surface soils exposed at Ft. Benning GA

---Infantry Training---
Troop Maneuvers
Individual Orienteering

--Longleaf Pines –
24-74 years
Vehicles & Infantry **Excluded**

Intensity of Disturbance

Approach:

Linear Discriminant Model Using 17 PLFA Variab

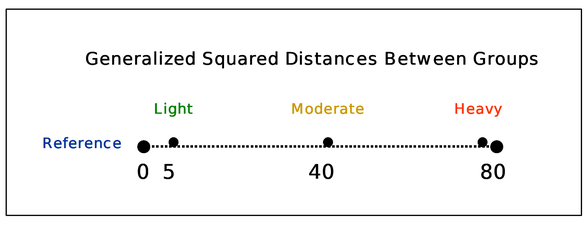
Non-Linear Artificial Neural Network

Results:

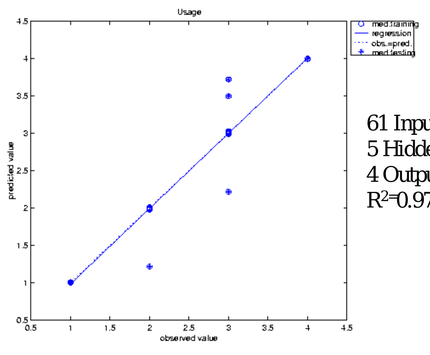
Linear Discriminant analysis showed that the reference and light transects were very similar while the moderate and heavy transects greatly differed in regards to the microbial community structure.

PLFAs included in the Discriminant Model

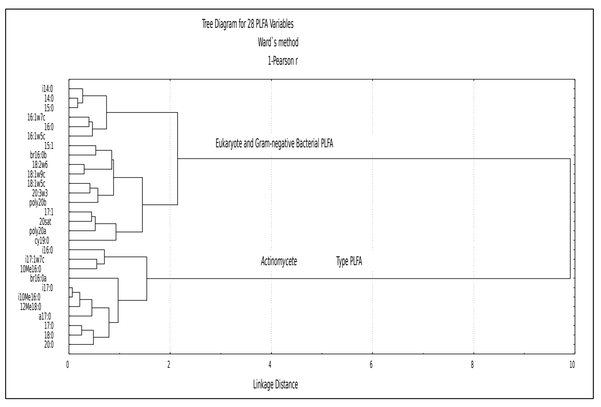
a15:0	i17:0	18:1w9c
i16:0	a17:0	18:0
16:1w7c	Cy17:0	10Me18:0
br16:0a	17:0	Cy19:0
i17:1w7c	i10Me16:0	20sat
10Me16:0	18:2w6	



Median Neural Network



61 Inputs (PLFA)
5 Hidden Nodes
4 Outputs
R^2=0.97



Conclusions:

The analysis based on the soil PLFA was successful, reflected above-ground changes, and provided an index of the degree of land disturbance (traffic) the soil received. Both linear discriminant and non-linear ANN analysis were able to adequately classify the degree of disturbance.

Analysis of Military Land Disturbance

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University of Florida

Microbial DNA Analysis

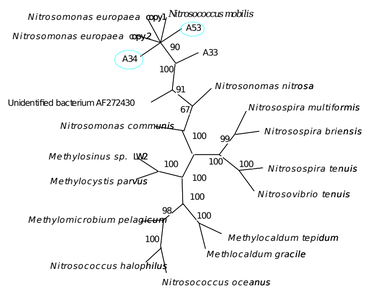
Objectives:

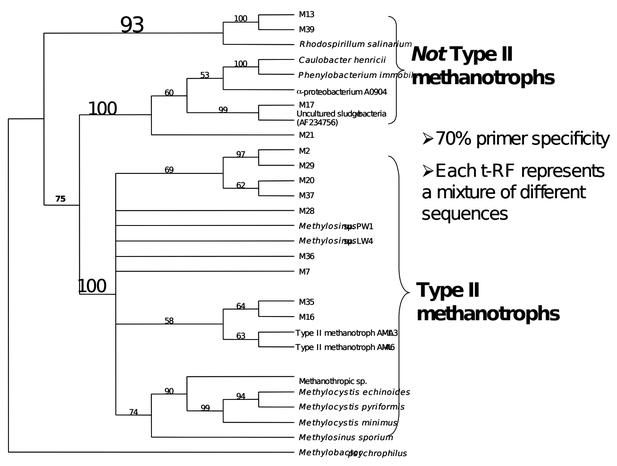
Evaluate the suitability of ammonia oxidizing bacteria as indicators of impact

Investigate potential relationships between soil properties and composition of these functional groups

Evaluate use of T-RFLP in these systems

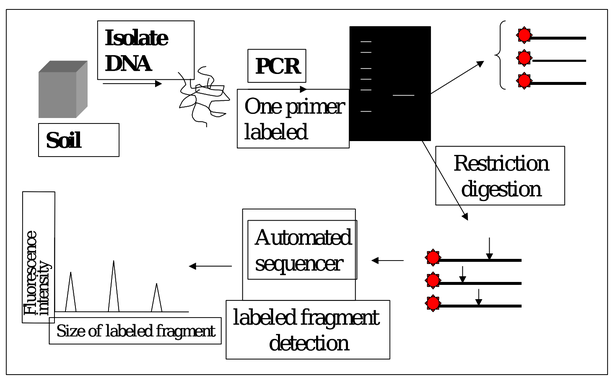
Phylogenetic Tree of AOB





- 70% primer specificity
- Each t-RF represents a mixture of different sequences

T-RFLP



Diversity index

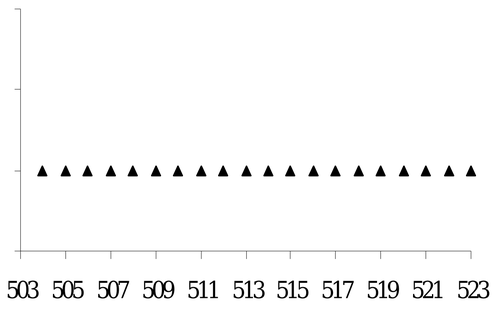


Table 1. Shannon Diversity Index of four different soils

	Wetland	Upland
High impact	0.40	0.23
Low impact	0.48	0.54*

*, Significant level $P<0.05$;
Shannon's Diversity Index $H=\text{Sum}(P_i \text{Log} P_i)$; i: 1 – S;
P_i: relative abundance of specific t-RF;
S: total number of t-RFs

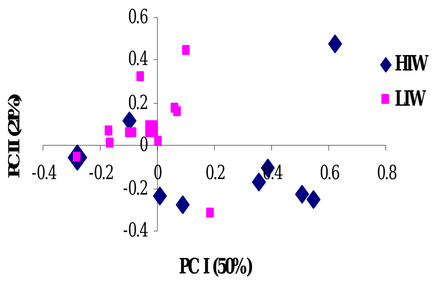


Table 2. Influence of Soil Parameters on Methanotrophs

Soil parameters	Relative abundance				Presence or Absence		
	30	147	160	179	147	160	179
pH							
Total C (%)							
Water extractable C							
Microbial biomass C			S			S	
Total N							
Microbial biomass N				S			S
Potentially mineralizable N					S		
NH_4-N				VS			
Total P	VS			VS			S
Mehlich P							
Oxalate extractable P	VS			S			S
Oxalate extractable							

S, significant $P<0.05$ & $P>0.01$; VS, very significant $P<0.01$.

Conclusions:

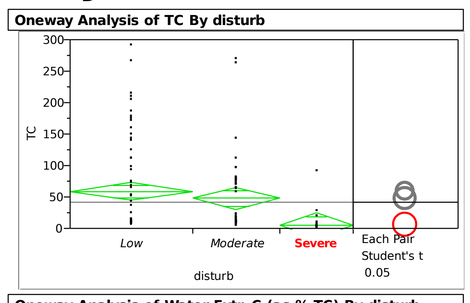
AOB assemblage composition may not be a suitable indicator
-Only one species of *N. europaea* detected
-One strain was characteristic of wetland soil but showed no relation to the degree of impact.

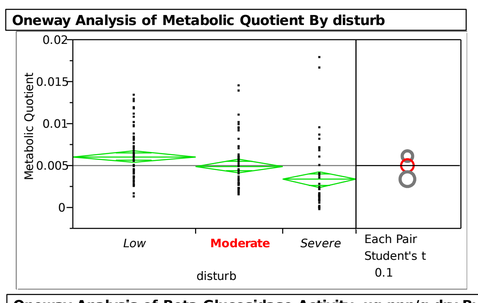
Methanotroph Assemblage Composition
-Diversity index discriminated between high and low impact upland soils
-PCII discriminated between high and low impact wetland soils
-Type II methanotroph assemblagemay be a potential indicator of soil quality at Ft. Benning
Differential influence of soil parameters on different soil methanotrophs

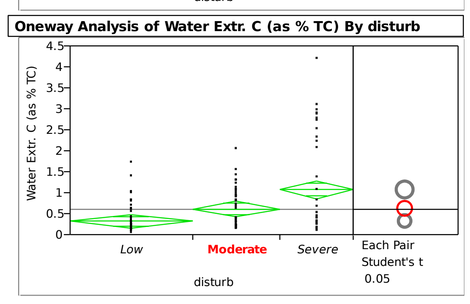
Soil Extracellular Enzymes and Microbial Activity

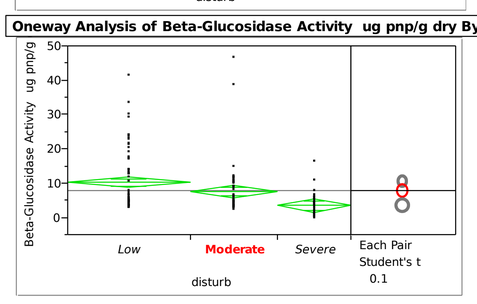
Soil microbial biomass, respiration and enzyme activity rates are potential indicators of nutrient (e.g. C, N, P) storage / cycling and organic matter decomposition rates in litter and soil/sediment layers. Correlating these parameters with chemical / physical measures of soil fertility may discern patterns of activity which integrate multiple properties of the soil. Military activity causes loss of soil structure and organic carbon, reducing microbial and enzymatic activities.

Figure 1. Comparison of Soil Carbon and Microbial Parameters. Means of Total Carbon (TC), Water Extractable Carbon (as % TC), Metabolic Quotient, and Beta-Glucosidase Activity, were compared for soil samples from transects of Low, Moderate and Severe impact. Metabolic Quotient is calculated as the ratio of Respiration rate (CO_2 production) and Microbial Biomass Carbon (MBC). Horizontal green line indicates mean height of diamonds indicate 95% confidence interval. Circles of different color on right of each graph indicate significant difference between means at $\alpha=0.05$.









While results indicate a strong inverse relationship between Total Carbon (TC) and the degree of soil disturbance, the percentage of TC that is in a water-extractable form (Water Extr. C) actually increases with increasing disturbance (Figure 1), reflecting changes in the partitioning of soil organic carbon. Metabolic quotient decreases with severity of impact, indicating less activity per unit of microbial biomass (Figure 1) and suggesting an increase in relative carbon availability (lability) in disturbed soils. Beta-glucosidase activity also decreases with impact level, indicating a general loss of substrate.

Table 1. ANOVA Models for Beta-glucosidase and Dehydrogenase Activity Levels. Analysis of variance for Beta-glucosidase and Dehydrogenase Activity levels indicate that TP, TC and PMN levels explain 91 and 94% of variance for these enzymes, respectively.

Beta-Glucosidase Activity
Model: BG=TP +TC +PMN +TC*TP

Analysis of Variance

Source	DF	Sum of Square	Mean Squar	F Ratio
Model	4	3433.1002	858.275	156.0843
Error	60	329.9275	5.499	Prob > F
C. Total	64	3763.0278		<.0001

Parameter Estimates

Term	Estimat	Std Erro	t Ratio	Prob>|t|
Intercept	4.3213198	0.486822	8.88	<.0001
TP	-0.026483	0.00861	-3.08	0.0032
TC	0.2141611	0.026199	8.17	<.0001
(TC-38.8312)*(TP-127.049)	-0.000204	0.000037	-5.50	<.0001
PMN	0.1966224	0.090834	2.16	0.0344

Summary of Fit

RSquare	0.912324
RSquare Adj	0.906479
Root Mean Square Error	2.34495
Mean of Response	8.527359
Observations (or Sum Wgts)	65

Dehydrogenase
Model: DH=TP +TC +PMN +TC*TP

Analysis of Variance

Source	DF	Sum of Square	Mean Squar	F Ratio
Model	4	4549618.3	1137405	258.3673
Error	60	264136.7	4402	Prob > F
C. Total	64	4813755.0		<.0001

Parameter Estimates

Term	Estimat	Std Erro	t Ratio	Prob>|t|
Intercept	214.56945	13.77448	15.58	<.0001
TP	-0.3369	0.243613	-1.38	0.1718
TC	7.6199075	0.741298	10.28	<.0001
(TC-38.8312)*(TP-127.049)	-0.01045	0.001049	-9.97	<.0001
PMN	3.2940874	2.570118	1.28	0.2049

Summary of Fit

RSquare	0.945129
RSquare Adj	0.941471
Root Mean Square Error	66.34967
Mean of Response	396.4371
Observations (or Sum Wgts)	65

Dehydrogenase activity is an indicator of general microbial activity (i.e. respiration). ANOVA analysis of Beta-glucosidase and Dehydrogenase levels (Table 1) indicate 91 and 94 % of variance are explained by Total Phosphorus (TP), TC, and Potentially Mineralizable Nitrogen (PMN) levels.

at Ft. Benning, GA

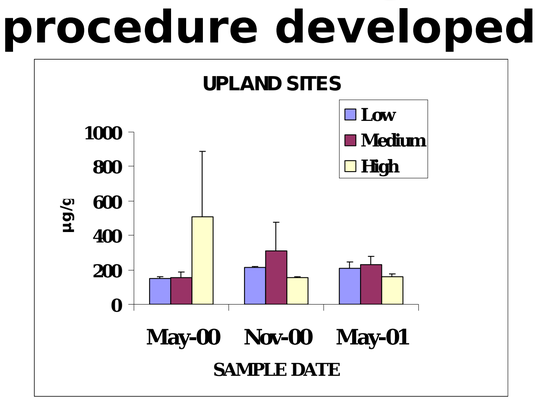
CS-85

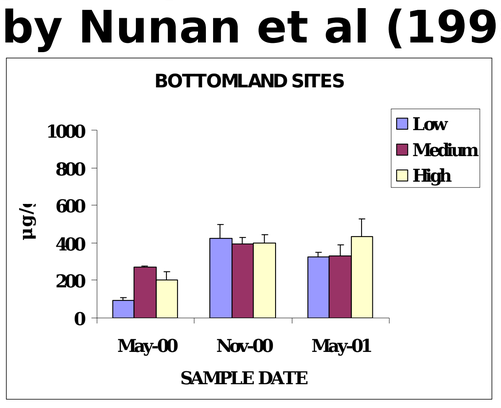
John Zak - Texas Tech University

Anthony Krzysik – Embry-Riddle University

Microbial Biomass Carbon and Fungal Functional Diversity

Methods; Six composite samples to 15 cm are collected from the three disturbance classes, low, medium, and high that occur with upland and bottom land sites within two locations along the Bonham Creek watershed. Microbial biomass is estimated using the fumigation extraction method of Vance et al (1987). The amount of microbial carbon is estimated using the spectrophotometer procedure developed by Nunan et al (1998).

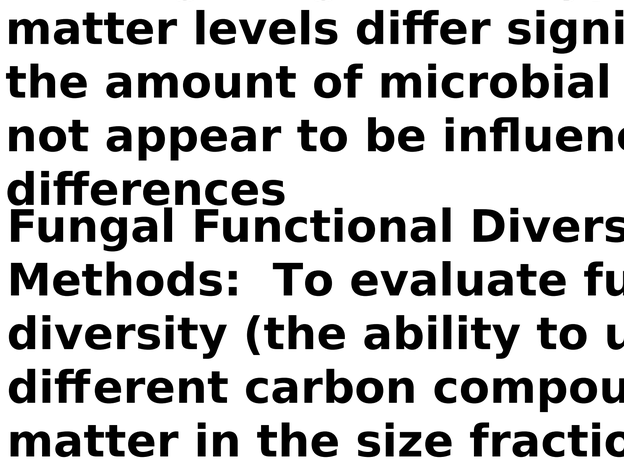


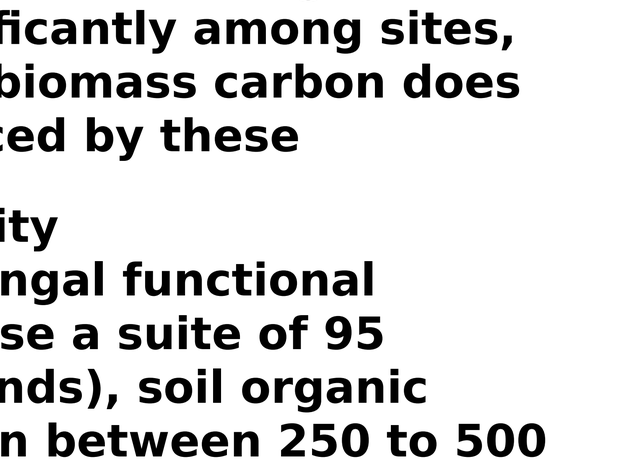


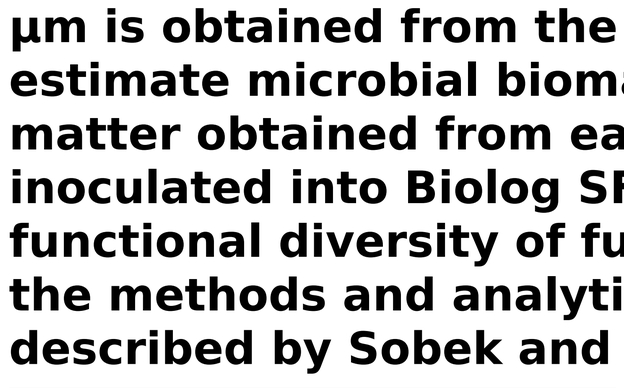
Results; There are no statistical differences in microbial biomass carbon between disturbance type sites for any sample period. Although vegetation type and soil organic matter levels differ significantly among sites, the amount of microbial biomass carbon does not appear to be influenced by these differences

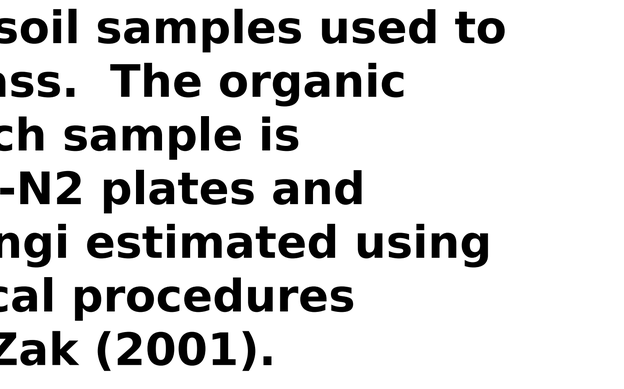
Fungal Functional Diversity

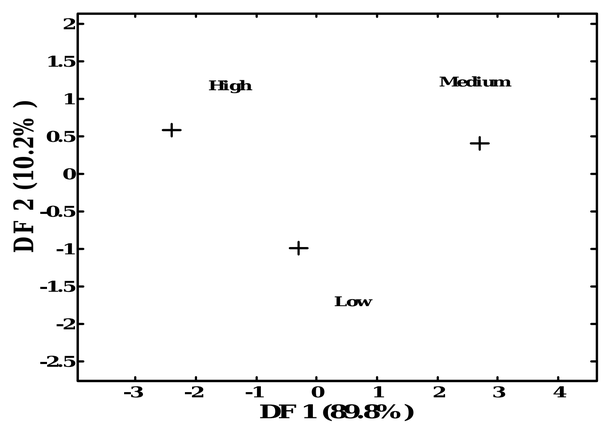
Methods: To evaluate fungal functional diversity (the ability to use a suite of 95 different carbon compounds), soil organic matter in the size fraction between 250 to 500 µm is obtained from the soil samples used to estimate microbial biomass. The organic matter obtained from each sample is inoculated into Biolog SF-N2 plates and functional diversity of fungi estimated using the methods and analytical procedures described by Sobek and Zak (2001).











Results: Fungal functional diversity is significantly influenced by disturbance type as reflected in the discriminant function analysis. In addition, season has a major impact on the fungal functional diversity with the high and medium disturbance classes exhibiting major shifts in their relationship to each other and to the low disturbance sites. Interestingly, fungal functional diversity of the low disturbance sites are not as influenced by season as are the medium and high disturbance sites.

References

Nunan, N., M. Morgan, and M. Herlgy, 1998. Ultrviolet absorbance (280nm) of compounds extracted from soil as a measure of microbial biomass. Soil Biol Biochem 30: 1599 – 1603.

Vance, E. D. P. C. Brookes, and D. S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass carbon. Soil Biol. and Biochem. 19: 703-707.

Sobek, E.J. C. Zak, and R. Strauss. Assessing soil fungal functional diversity: method devleopment and analytical procedures (in preparation).

Overall Results and Conclusions

-Soil microbial assessment performed by four different research groups using different methods (DNA, PLFA, Soil Enzymes, and Functional Diversity) generally agree

- The soil microbial community reflects aboveground disturbance impacts

-Key soil microbial parameters may be used as a measure of the degree of disturbance

-This information may be used to aid in the management of military lands